Expert Opinion

Monthly Focus: Biologicals, Immunologicals & Drug Delivery

Somatostatin receptor agonists and antagonists

A Michael Crider
School of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209-0470, USA

Somatostatin is a cyclic peptide that is widely distributed in the CNS, the periphery and in a variety of tumours. Two biologically active forms, somatotropin release-inhibiting factor (SRIF)-14 and SRIF-28, exert their effects through activation of five G-protein-coupled receptor subtypes (sst_1 – sst_5). These peptides act as neurotransmitters or hormones and inhibit the secretion of other peptides, such as insulin, growth hormone and glucagon. Metabolically stable peptide and structurally diverse non-peptide analogues have been developed as subtype-selective agonists and antagonists. The availability of these novel SRIF analogues will greatly facilitate our understanding of the function and role of specific SRIF receptors. SRIF analogues offer therapeutic potential in the regulation of hormone secretion, diseases of the CNS and periphery and in the treatment and diagnosis of various tumours. This review will focus on an overview of SRIF, new developments related to SRIF role and function and the discovery of novel peptide and non-peptide agonists and antagonists.

Keywords: somatostatin, somatostatin receptors (sst), somatotropin release-inhibiting factor (SRIF), SRIF agonists, SRIF antagonists

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1. Introduction

1.1 Endogenous peptides

Somatostatin (somatotropin release-inhibiting factor [SRIF]; compound 1) is a cyclic tetradecapeptide that was isolated from hypothalamic extracts by Brazeau *et al.* [1]. SRIF was shown to exist in the two biologically active forms SRIF-14 and SRIF-28, an extended form of 14 amino acids from the *N*-terminal end of SRIF-14. Both forms are derived from the polypeptide precursor presomatostatin and they contain one internal disulfide bond between residues 3 and 14. These peptides may function as neurotransmitters or hormones.

Cortistatin-14 (CST-14; compound 2) is a recently discovered neuropeptide from rat brain that has 11 of the 14 amino acids in common with SRIF-14 [2]. The name of this peptide is derived from its predominate cortical expression and its ability to depress cortical activity [3]. Subsequent studies by Fukusumi *et al.* [4] demonstrated that a human preprocortistatin (PPCST) leads to a 17 amino acid peptide, CST-17. Binding studies in CCL-39 (Chinese hamster lung fibroblast) cells demonstrated that both SRIF-14 and CST-14 have a high affinity for SRIF receptors. None of the SRIF receptor subtypes showed any selectivity for either SRIF-14 or CST-14 [5]. The post-translational processing of PPCST from rat brain was studied in AtT-20 cells. Analysis of the processed products revealed that CST-14, CST-29 (an extended form of 15 amino acids from the *N*-terminal end of CST-14) and unprocessed PPCST were produced in a ratio of (41:45:55). However, CST-14 was the predominately-released product under basal- and forskolin-induced experimental conditions [6]. Another recent study demonstrated that CST-14, but not SRIF, binds to growth hormone secretagogue receptors (GSHRs) from the human pituitary. These workers

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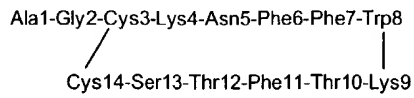
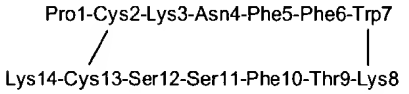


1 SRIF-14



2 CST-14

speculated that CST-14 may play a modulatory role on GSHR activity in the human pituitary [7]. Dalm *et al.* [8] used reverse transcription-polymerase chain reaction (RT-PCR) techniques to investigate the expression of SRIF and CST in a variety of human lymphoid tissues and immune cells. SRIF was found only in the human thymus, whereas CST mRNA was found in the lymphoid tissues, immune cells and bone marrow. Furthermore, CST mRNA was shown to be upregulated during differentiation of monocytes into macrophages and dendritic cells. Using radioaudiography, CST displaced [^{125}I]-Tyr3 octreotide in human thymic tissues and on SRIF receptor-expressing cells. This study suggests that CST may be the endogenous ligand at SRIF subtype 2 receptors in the human immune system. However, the question remains unanswered as to whether specific CST receptors exist.

1.2 SRIF receptor subtypes

SRIF exerts its effects by binding to a family of widely distributed, structurally related SRIF receptors (ssts). Five subtypes of receptors have been cloned and identified. The receptors belong to the G-protein-coupled superfamily and are designated sst_1 – sst_5 [9,10]. On the basis of structural and pharmacological aspects, sst_2, sst_3 and sst_5 comprise the $SRIF_1$ receptor family, while the sst_1 and sst_4 subtypes comprise the $SRIF_2$ receptor family [9,11]. These major families of SRIF receptors are distinguished on the basis of the increased affinity of the $SRIF_1$ family for hexapeptide and octapeptide analogues of SRIF [12]. Two isoforms of the sst_2 receptor that differ only in their intracellular C termini are found in the mouse and rat. These splice variants are designated sst_{2A} and sst_{2B}, respectively [13,14].

G-protein-linked second messenger systems, including adenylyl cyclase, Ca^{2+} and K^+ ion channels, phospholipase C (PLC) and mitogen-activated protein kinase (MAPK), have been implicated in the signal transduction of SRIF receptors [15,16]. Agonist binding at ssts results in potent inhibition of adenylyl cyclase. SRIF receptor signalling also causes induction of several phosphatases, such as the serine–threonine phosphatases [17]. Other signalling pathways for ssts include phospholipase A_2-dependent stimulation of arachidonate production in hippocampal neurons and PLC-mediated stimulation of inositol-1,4,5-triphosphate (IP_3) formation in astrocytes and smooth muscle cells [18,19].

Recent studies suggest that individual receptor subtypes can activate more than one G protein and G-protein-linked signalling cascade. The fact that single cells express more than one receptor subtype has presented perplexing questions such as whether multiple ssts in the same cell are redundant or whether they coordinate functionally for greater signalling diversity. SRIF receptor subtypes sst_{2A} and sst_3 were shown to exist as either homodimers or as heterodimers when expressed in human embryonic kidney (HEK)-293 cells. Although the sst_{2A}–sst_3 heterodimer exhibited properties of the sst_{2A} receptor subtype, the heterodimer was devoid of sst_3 receptor function [20]. A subsequent study showed that the sst_{2A} receptor forms a stable heterodimer with the μ-opioid receptor (MOR1). Unlike the situation with the sst_{2A}–sst_3 heterodimer, the sst_{2A}–MOR1 heterodimer retained ligand binding and coupling properties of both receptors. Interestingly, heterodimerisation promoted cross-modulation of phosphorylation, internalisation and desensitisation of these receptors [21]. These workers speculated that heterodimerisation might be a means of modulation of phosphorylation and desensitisation of G-protein-coupled receptors.

1.3 Distribution and physiological functions of somatostatin receptors

The five SRIF receptor subtypes are found in the CNS, periphery and in various tumours [17]. All five receptor subtypes are expressed in the brain. Areas rich in the expression of each individual subtypes genes are the cerebral cortex for subtypes 1 and 2, amygdala for subtypes 1 and 3 and the hypothalamus and preoptic area for subtype 5 [22,23]. Compared with the other subtypes, the expression of subtype 4 in the brain is relatively poor [24]. The sst_2 and sst_5 receptors are the major subtypes found in human pituitary cells, with a predominant expression of sst_5 [25]. The ssts are present in the endocrine and exocrine pancreas and on growth plates of long bones [26]. The human stomach expresses all five subtypes, whereas only subtypes 1 – 4 have been identified in the rat stomach [27]. The rat small intestine displays moderate levels of sst_1 and sst_5, low levels of sst_3 and sst_4, but no expression of sst_2 [17,28]. The adrenal glands display high levels of sst_2 and moderate levels of sst_1 and sst_3. In addition, ssts are found in the liver and spleen (sst_3) and in the lung and heart (sst_4) [16]. Recent studies have shown the presence of ssts in human vascular tissue. Human blood expresses high levels of sst_1 with much less expression of sst_2 and sst_4. Subtypes 3 and 5 were not detected in either normal or diseased blood vessels [29]. SRIF receptor subtypes 3, 4 and 5 are found in mouse proximal tubules, while subtypes 1 and 2 have been found in rat or human kidneys, primarily in the collecting ducts [30].

Specific physiological functions have only been attributed to SRIF receptor subtypes 2 and 5. The release of growth hormone (GH) from primary cultures of anterior pituitary was

shown to involve both sst_2 and sst_5 [31]. Culler *et al.* [32] showed that SRIF subtypes 2 and 5 are responsible for suppression of GH release in the human pituitary. Using primary cultures of cells from acromegalic patients, these workers showed that individuals who failed to respond fully to SRIF analogue therapy had dramatically reduced sst_2 receptor expression. Strowski *et al.* [33] used SRIF receptor 2 knockout (SSTR2 KO) mice to show that glucagon release in mouse islets was primarily mediated by sst_2, whereas insulin secretion was regulated by sst_5. A subsequent study [34] using SRIF receptor 5 knockout (SSTR5 KO) mice, demonstrated that sst_5 mediates SRIF inhibition of pancreatic insulin secretion and plays an important role in the regulation of glucose homeostasis and insulin sensitivity. Subtype-selective analogues were used to identify the ssts involved in regulation of adrenocorticotropic hormone (ACTH) release from AtT-20 cells, a model for cell line pituitary corticotropes. SRIF and non-peptidyl sst_2 agonists were shown to inhibit forskolin- and corticotropin-releasing hormone (CRH)-induced increases in intracellular cAMP. Also, non-peptide sst_2- and sst_5-selective agonists potently inhibited CRH-induced ACTH release using this model [35].

Glucagon-like peptide (GLP)-1 is a postprandial proglucagon-derived peptide that is released from the endocrine cells of the gastrointestinal tract. This peptide acts to lower blood glucose levels by stimulation of insulin release from pancreatic islet β-cells [36]. In a rat insulinoma cell line R1Nm5F model, GLP-1 stimulated insulin release, cell proliferation and increased intracellular cAMP concentrations. The R1Nm5F cell line primarily contains sst_1 and sst_2 receptors. Administration of selective sst_1 and sst_2, but not sst_3, selective non-peptidyl agonists or a peptide SRIF analogue returned GLP-1-stimulated insulin release, cell proliferation and intracellular cAMP to baseline levels [37]. Using a fetal rat intestinal culture, Chisholm and Greenberg [38] demonstrated that SRIF-28 dose-dependently inhibited GPL-1 secretion which was stimulated by gastrin-releasing peptide. This inhibition was more potent with SRIF-28 than with SRIF-14. Conversely, GLP-1 increased SRIF-14 and SRIF-28 by 5- and 3-fold, respectively. The sst_5-selective peptide analogue BIM-23268 was almost as effective as SRIF-28 at inhibiting GPL-1 secretion. The results of this study suggest that GLP-1 inhibition is regulated by the action of SRIF-28 on sst_5 receptors. The authors concluded that GLP-1 secretion is autoregulated by sst_5 activation and that a feedback loop exists between GLP-1 and SRIF-28 that is under sst_5 control.

Ghrelin, a 28 amino acid peptide, is a natural ligand at the GHSR that strongly stimulates the secretion of GH in both animals and humans. In a recent study in human volunteers, SRIF-14 was shown to almost completely suppress GH-releasing hormone (GHRH)-induced GH release [39]. A subsequent study by these workers evaluated the effects of intravenous administration of SRIF-14 or CST-14 on ghrelin, GH and insulin and glucose levels in young men. Both CST-14 and SRIF-14 inhibited circulating levels of ghrelin by ~ 55%. The ghrelin levels remained lower than baseline values even after the 30-min infusion of SRIF-14 or CST-14 was terminated [40].

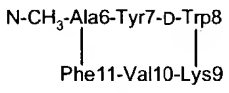
SRIF analogues were found to bind with high affinity to rabbit retinal membranes. Furthermore, SRIF-14 and subtype-2-selective agonists were shown to stimulate GTPase activity in a concentration-dependent manner. The results of this study suggested that SRIF produces its effects in the rabbit retina by binding to sst_2 receptors that are functionally coupled to a $G_{o\alpha}$ protein [41]. In a study using retinae from SSTR2 KO mice, SRIF-14 and its analogues failed to alter glutamate release. However, in wild-type (WT) mice, SRIF-14 and its analogues displayed high selectivity for sst_2 in mouse retinae and also inhibited potassium-evoked release of glutamate [42]. Studies using the rat retina showed that SRIF-14 and an sst_2-selective non-peptide agonist stimulated nitric oxide production, whereas an sst_2-selective antagonist blocked this effect. These studies provide strong evidence that SRIF-14 regulates nitric oxide synthesis in the rat retina through activation of subtype 2 receptors [43].

SRIF influences carcinogenesis, tumour growth and tumour metastasis through a combination of direct and indirect mechanisms [16]. This peptide inhibits cell secretion and prevents cell proliferation by inducing cell cycle arrest or apoptosis. Most human tumours express one or more SRIF receptors that are responsive to SRIF or its analogues. Although the direct effects are mediated through ssts, SRIF and its analogues may exert an indirect effect by inhibiting hormone and growth factor secretion, angiogenesis and immune cell activity in non-tumour cells [17]. Reubi *et al.* [44] used autoradiography with [^{125}I]-Tyr-[Leu8,D-Trp22,Tyr25] SRIF-28 and its displacement with receptor-selective ligands to study sst receptor protein expression in approximately 200 tumour types. This study showed that sst_2 was expressed in a majority of tumours. Teijeiro *et al.* [45] showed that HL-60 cells contained sst_2 receptors and that treatment with an SRIF-14 analogue resulted in increased cell death through apoptosis. This effect was attributed to activation of subtype 2 receptors through a mechanism independent of p53. In a project to study the localisation of SRIF and ssts in benign and malignant ovarian tumours, Hall *et al.* [46] found that SRIF correlated well with the expression of sst_1, sst_2, sst_3 and sst_5 receptors in epithelial, vascular and stromal compartments of these tumours. Recently, Hansson *et al.* [47] reported, for the first time, the presence of sst_2 and sst_4 mRNA in benign prostatic hyperplasia (BPH) and malignant cells of prostate cancer tissue. This research showed that sst_2 and sst_4 receptors were upregulated in malignant cells, leading to speculation that subtype 2- and 4-selective agonists could have therapeutic potential in the treatment of prostate cancer.

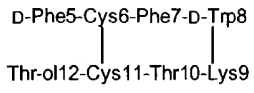
2. Somatotropin release-inhibiting factor analogues with affinity for somatostatin receptors

2.1 Peptide agonists

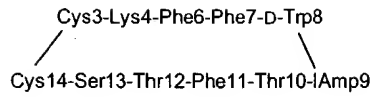
Poor bioavailability and rapid inactivation by peptidases severely limit the therapeutic effectiveness of SRIF. Thus, the



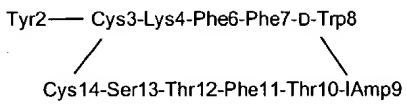
3 MK-678



4 Octreotide



5 CH-275



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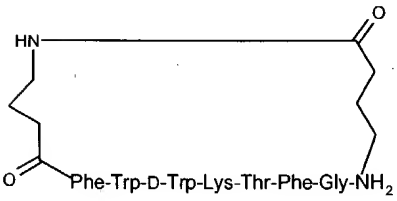


7 PTR-3173

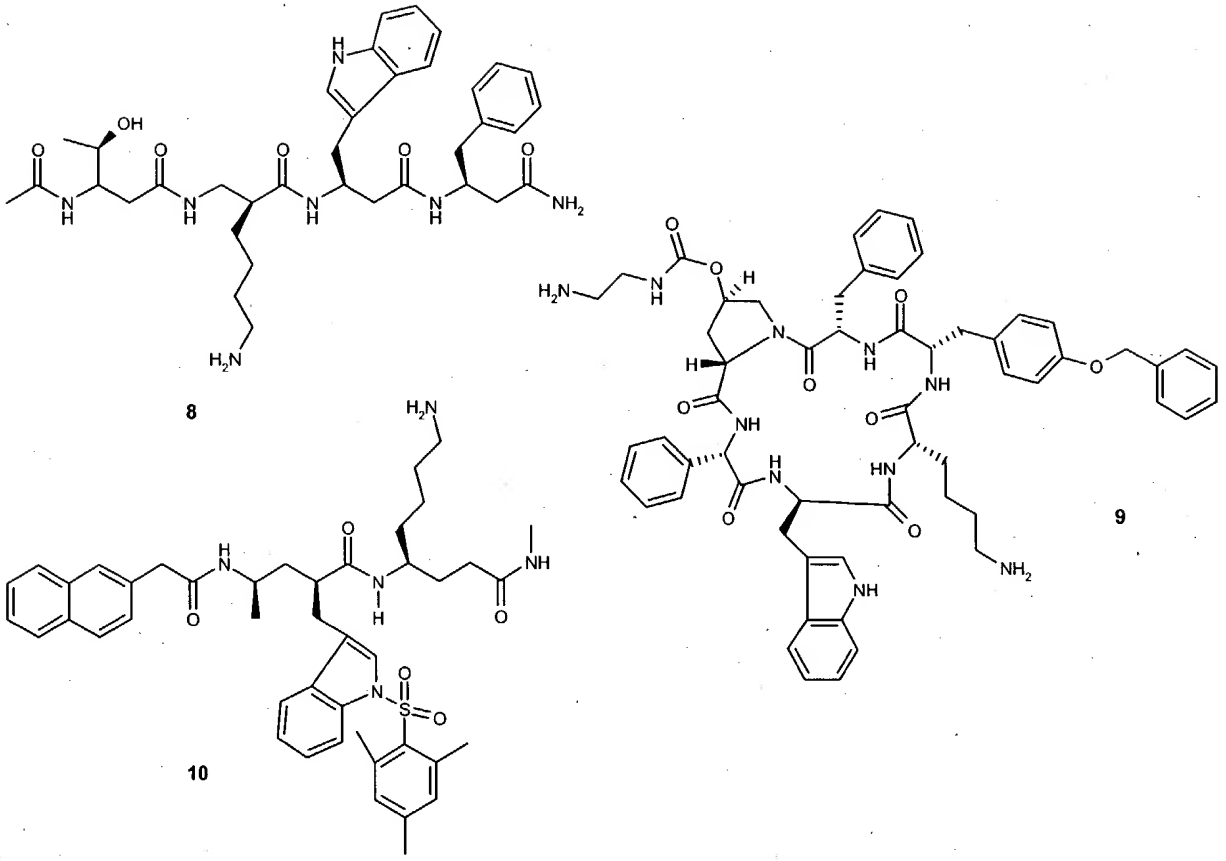
search for metabolically stable peptidomimetics has been the focus of extensive research [48]. Structure–activity relationship (SAR) studies have shown that the Trp8 and Lys9 residues are essential for biological activity and that the tetrapeptide Phe7-Trp8-Lys9-Thr10 comprises the critical β-turn of SRIF [49]. This information led to the development of the hexapeptide MK-678, compound 3 [50], and the cyclic octapeptide octreotide (SMS-201955; compound **4**) [51], both potent SRIF agonists. The des-amino acid (1,2,5)[D-Trp8,IAmp9], CH-275 (compound 5) and its [^{125}I-Tyr2] analogue, compound 6, were shown to bind with high affinity and selectivity at sst_1 receptors in displacement studies using [^{125}I-Tyr11]-SRIF with dissociation constant (K_d) values of 1.8 ± 0.7 nM and 0.5 ± 0.1 nM, respectively [52]. Additional studies from these researchers demonstrated that des-amino acid1,2,5[D-Trp8,IAmp9,[^{125}ITyr11]-SRIF and des-amino acid1,2,5[D-Trp8,IAmp9,[^{125}ITyr11]-Cbm-SRIF bound at sst_1 receptors with median inhibitory concentration (IC_{50}) values of 17.1 ± 6 nM and 8.1 ± 1.3 nM, respectively. Furthermore, these analogues were shown to detect sst_1-expressing tumours using *in vitro* receptor autoradiography [53]. These analogues were shown to be agonists since they inhibited forskolin-induced cAMP accumulation.

Rajeswaran *et al.* [54] carried out an *N*-methyl scan on the cyclic octapeptides, D-Phe5-c[Cys6-Phe7-D-Trp8-Lys9-Thr10-Cys11]Thr12-NH_2 (SRIF numbering, K_d 17.9 ± 2.5 nM, sst_3) and Tyr5-c[Cys6-Phe7-D-Trp8-Lys9-Thr10-Cys11]-Thr12-NH_2, in an attempt to block intramolecular hydrogen bonding sites and to increase the metabolic stability of these peptides. Displacement studies using cell membranes of Chinese hamster ovary (CHO)-K1 cells showed that *N*-methylation of Phe7, Thr10, Cys11 and Thr12 essentially eliminated binding affinity, whereas *N*-methylation of Tyr5 or D-Phe5 or Cys6 resulted in analogues with K_d values of < 20 nM at sst_3 receptors.

A long-acting cyclic SRIF analogue (c[GABA-Phe-Trp-D-Trp-Lys-Thr-Phe-GlyC3-NH_2], PTR-3173; compound 7) was recently described [55]. This novel peptide showed 1000- and 10,000-fold more potent *in vivo* inhibition of GH release compared with inhibition of glucagon or insulin secretion, respectively. PTR-3173 bound with high affinity for sst_2 (3 ± 0.75 nM), sst_4 (7 ± 1.1 nM) and sst_5 (1 ± 0.05 nM). Furthermore, PTR-3173 is reported to be the first SRIF analogue that demonstrates selective *in vivo* inhibition between GH and insulin release. As an extension of this work, Gazal *et al.* [56] replaced the lactam bridge in PTR-3173 by a disulfide bond. This analogue exhibited excellent metabolic stability but a different receptor selectivity for sst_2 (28.7 ± 2.17 nM), sst_3 (15.6 ± 2.1 nM), sst_4 (> 1000 nM) and sst_5 (1.3 ± 0.08 nM).

Gademann *et al.* [57] designed and synthesised nonconstrained, linear β-peptides which incorporate the critical β-turn (Phe7-Trp8-Lys9-Thr10) of SRIF. The *N*-acetylamide (Acβ3-HThr-β3-HLys-β3-HTrp-β3-HPhe-NH_2; compound **8**) exhibited a K_d at sst_4 of 83 nM and at least a 100-fold selectivity with respect to other ssts. Movement of the Lys moiety to the 3-position resulted in a decrease in sst_4 affinity by more than 1000-fold.

Unlike most previous studies to develop subtype-selective SRIF analogues, Reubi *et al.* [58] developed agonists that bind equally well to all SRIF receptor subtypes. The rationale being that such analogues could offer a therapeutic advantage in the treatment of multiple sst receptor-expressing tumours. The target compound of this investigation (H_2N-Tyr-c[D-Dab-Arg-Phe-Phe-D-Trp-Lys-Thr-Phe]; KE-108) exhibited high affinity for all SRIF receptor subtypes (sst_1 = 2.6 ± 0.4 nM, sst_2 = 0.9 ± 0.1 nM, sst_3 = 1.5 ± 0.2 nM, sst_4 = 1.6 ± 0.1 nM and sst_5 = 0.65 ± 0.1 nM). The related 3-I-Tyr analogue of KE-108, KE-119, also demonstrated high affinity, albeit slightly lower affinity, at all ssts. These analogues act as agonists as shown by their ability to inhibit forskolin-stimulated cAMP production. The presence of the Tyr in position zero of the cyclic peptide offers significant utility in the development of radioactive analogues having the potential to identify tissues expressing sst_1 – sst_5 receptor subtypes. Researchers at Novartis [101] reported the development of a cyclic hexapeptide (compound **9**) with a similar broad binding profile at ssts. This peptide analogue exhibited IC_{50} values of 9.3, 1, 1.5, > 100 and 0.16 nM at sst_1 – sst_5, respectively. The compound was stated to inhibit GH and insulin-like growth factor (IGF)-1 release in



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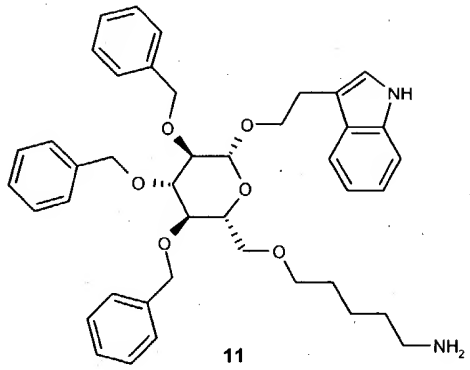
rats. Furthermore, this analogue inhibited tumour growth in a subcutaneous tumour model in nude mice.

Seebach *et al.* [59] synthesised a series of simple γ-dipeptide derivatives for sst receptor binding affinity. These derivatives have the Trp side chain in the γ2-position of the first amino acid and the Lys moiety at the γ4-position of the second amino acid. The γ-dipeptide **10** showed moderate affinity for subtypes 1, 3 and 5 using a CCL-39 cell line. This work demonstrates that a simple γ-dipeptide can mimic SRIF, and these γ-dipeptides may be potentially useful in the development of metabolically stable peptidomimetics of SRIF.

A patent filed by researchers at Tulane [102] described the solid-phase synthesis of 18 cyclic peptides with affinity for ssts. The *N*-methylated analogue, [N-CH_3-D-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH_2, exhibited K_d values of 316 nM for sst_1, 1.03 ± 0.26 nM for sst_2, 17.9 ± 2.5 nM for sst_3, > 1000 nM for sst_4, and 4.89 ± 1.4 nM for sst_5. This analogue was shown to act as an agonist in a rat pituitary cell assay with an IC_{50} value of 0.32 nM in inhibition of GH release. These same researchers at Tulane [103] filed a patent on cyclic hydrophilic peptides for use in tumour detection and antitumour therapy. These analogues were rendered more hydrophilic by incorporating either a D-Lys-Lys or a D-Arg-Arg around a Tyr residue in the peptide backbone. One of the analogues (D-Lys-Tyr-Lys-Tyr-Lys-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH_2; JF-0559) showed K_d values of 0.3 nM for sst_1, 4.1 nM for sst_2, 6.4 nM for sst_3, 515 nM sst_4 and 13.4 nM for sst_5, respectively. This analogue exhibited an IC_{50} value of 0.3 nM in a rat pituitary cell assay to assess agonist activity (sst_2-mediated) on GH release. These multiple Tyr-containing peptides are claimed to have a broader receptor-binding profile than previous derivatives. Also, the D-Tyr-containing analogues have high receptor affinity and the D-iodo-Tyr derivatives are more stable toward metabolic degradation than the corresponding L-analogues. Therefore, the D-iodo-Tyr derivatives retain the label for a longer period than the L-isomers.

2.2 Peptide antagonists

The development of antagonists at ssts has lagged considerably behind the discovery of sst receptor agonists. The availability of selective antagonists is essential to fully characterise the functions of the individual SRIF receptor subtypes. Bass *et al.* [60] reported the first sst receptor antagonist in 1996. This cyclic octapeptide (AcNH-4-NO_2Phe5[D-Cys6-Tyr7-D-Trp8-Lys9-Thr10-Cys11]-D-Tyr12-NH_2; CYN-154806) inhibits the binding of SRIF at sst_2 and sst_5 receptors and antagonises SRIF agonist-stimulated inhibition of cAMP. Inversion of the chirality of disulfide-bridged octapeptide SRIF agonists at positions 5 and 6 (SRIF numbering: D5, L6 to L5, D6) led to the discovery of DC-3848, H-Nal-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Nal-NH_2, a selective antagonist at sst_2 receptors [61]. Additional



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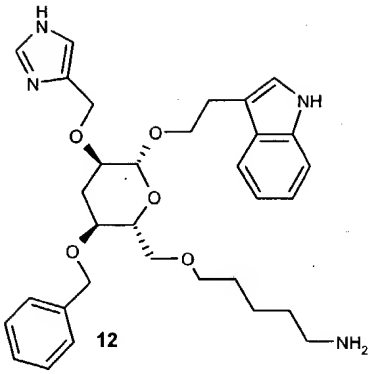

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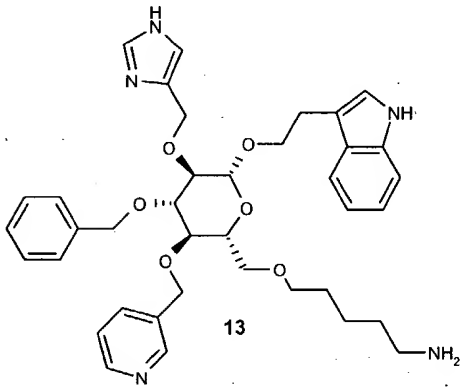

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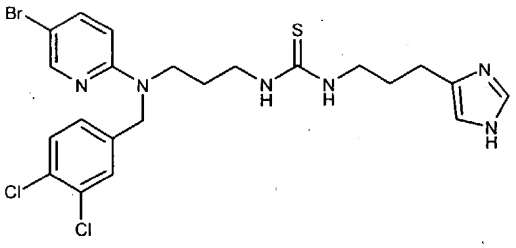
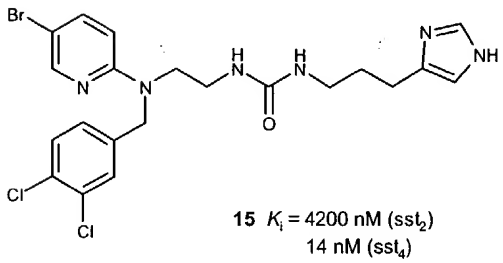
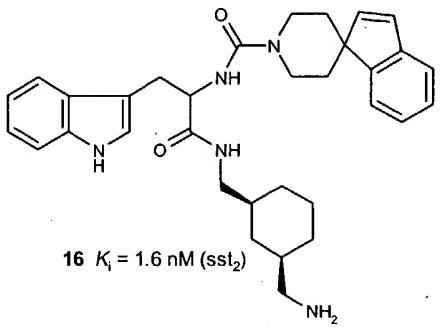


14 NNC 269100
K_i = 621 nM (sst_2)
6 nM (sst_4)

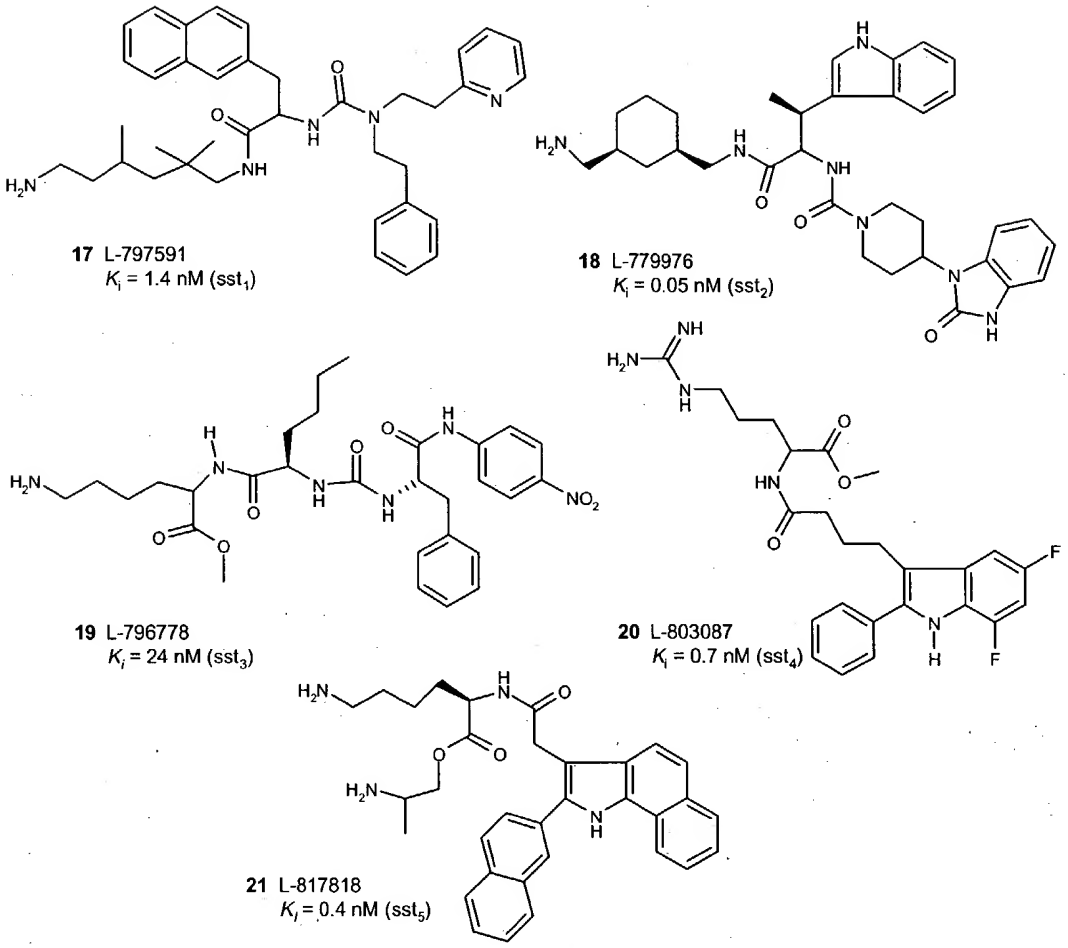


15 K_i = 4200 nM (sst_2)
14 nM (sst_4)



16 K_i = 1.6 nM (sst_2)

studies from this group led to the cyclic octapeptide antagonist, H-Cpa-c[D-Cys6-Tyr7-D-Trp8-Lys9-Thr10-Cys11]-Nal-NH_2, an analogue with a dissociation constant for an inhibitor (K_i) of 26 nM at sst_2 receptors. This analogue showed potent antagonistic effects to SRIF in an *in vitro* rat pituitary assay [62]. Using a procedure similar to that described for the development of peptide agonists, Rajeswan *et al.* [63] carried out a systematic *N*-methylation approach of the lead antagonist, (H-Cpa-c[D-Cys6-Tyr7-D-Trp8-Lys9-Thr10-Cys11]-Nal-NH_2), to produce several antagonists with high affinity for sst_2, sst_3 and sst_5. The derivative containing an *N*-CH_3-Lys9 residue showed slightly lower binding affinity at sst_2, but this analogue was about fourfold more potent in an *in vitro* GH release assay. This analogue also inhibited sst_5 receptor calcium mobilisation in an *in vitro* assay in CHO-K1 cells. Replacement of the Lys9 residue by a 2,4-diaminobutyric acid group led to a compound with high selectivity at subtype 3 receptors. Further investigation of these SRIF antagonists led to sst_2 antagonists with high affinity for subtype 2 receptors. These same workers described related compounds in a 2002 patent [104]. The cyclic octapeptide Cpa-c[D-Cys-Tyr-D-Trp-*N*-CH_3Lys-Thr-Cys]-Nal exhibited K_i values of 1000 nM for sst_1, 5.51 ± 1.85 nM for sst_2, 115.1 ± 16.9 nM for sst_3, 1000 nM for sst_4, and 70.7 ± 25.8 nM for sst_5. Substitution of a hydrophilic His for the Nal12 at the C terminus resulted in a highly potent and selective sst_2 antagonist Cpa-c[D-Cys-Pal-D-Trp-*N*-CH_3Lys-Thr-Cys]-His-NH_2. An *in vitro* rat pituitary cell culture assay that measured inhibition of SRIF-inhibited GH release [64] was utilised to determine antagonistic activity of these compounds.



17 L-797591
K_i = 1.4 nM (sst_1)

18 L-779976
K_i = 0.05 nM (sst_2)

19 L-796778
K_i = 24 nM (sst_3)

20 L-803087
K_i = 0.7 nM (sst_4)

21 L-817818
K_i = 0.4 nM (sst_5)

Reubi *et al.* [65] described the synthesis and evaluation of a family of disulfide-bridged octapeptide sst_3 receptor antagonists. The des-AA1,2,4,5,12,13-SRIF analogue, carbamoyl-desAA1,2,4,5,12,13[D-Cys3,Tyr7,D-AgI8 (CH_3, 2-naphthoyl)-SRIF, sst_3-ODN-8] exhibits comparable affinity to SRIF-28 at subtype 3 receptors. This analogue reversed SRIF-28-induced inhibition of forskolin-stimulated cAMP accumulation and antagonised SRIF-28-induced PLC stimulation. The Tyr7 residue was labelled with ^{125}I to yield [^{125}I-Tyr7]sst_3–ODN-8, which was shown to label sst_3-transfected CCL-39 cells.

In a recent report, researchers at Novartis evaluated the D-Tyr8 and L-Tyr8 isomers of CYN-154806 in recombinant human SRIF receptors and endogenous guinea-pig ileum receptors [66]. Radioligand-binding studies demonstrated nanomolar affinity at sst_2 receptors with 40- to 4500-fold greater affinity than at sst_1, sst_3 or sst_5 receptors. The L-isomer of CYN-154806 also demonstrated high affinity for subtype 5 receptors. Interestingly, both the D- and L-isomers demonstrated full agonist activity in inhibitory forskolin-induced cAMP accumulation. In the guinea-pig assay, the L-isomer showed agonist activity, whereas the D-isomer was inactive. This work implies that CYN-154806 is not a selective sst_2 antagonist and that the D- and L-Tyr8 isomers have high affinity for sst_5 receptors. Furthermore, CYN-154806 was shown to elicit agonist activity at sst_2.

2.3 Non-peptide agonists

The development of orally-active, non-peptide SRIF analogues with high selectivity for ssts has been an area of intense investigation in the last few years [11,67]. Hirschmann *et al.* [68] described the first non-peptide SRIF agonist in which a D-glucose scaffold was utilised to mimic the critical β-turn of SRIF. In compound **11**, the key pharmacophoric groups occupy similar spatial orientations as found in octreotide; however, the compound demonstrated only weak agonist activity in AtT-20 cells. Additional studies from this group [69] demonstrated that compound 12 exhibited potent affinity for sst_4 receptors. Modification of compound 12 by the introduction of a 3-picolyl at C-4 and an (imidazol-4-yl)methyl group at C-2 led to compound **13**. This analogue binds with high affinity and selectivity at sst_4 [70].

Ankersen *et al.* [71] reported the first non-peptide with high affinity for cloned human sst_4 receptors in 1998. The thiourea (**14**, NNC-269100) exhibited K_i values at sst_2 and sst_4 of 621 and 6 nM, respectively. Replacement of the

thiourea moiety in compound **14** by a urea function resulted in the sst_4-selective analogue **15**. This compound bound at sst_2 and sst_4 receptors with K_i values of 4200 and 14 nM, respectively. In this case, the urea moiety leads to retention of sst_4 affinity but with enhanced selectivity versus subtype 2 receptors. Compounds **14** and **15** showed full agonist activity and potently inhibited cAMP accumulation with median effective concentration (EC_{50}) values of 26 and 24 nM, respectively [72].

Yang *et al.* [73] reported the first non-peptide with high affinity and selectivity at human SRIF subtype 2 receptors. Compound **16** was a full agonist that potently inhibited forskolin-stimulated cAMP accumulation with an IC_{50} of 2 nM [74]. Additional work from the Merck group using combinatorial synthetic methods and high-throughput screening led to the discovery of non-peptides **17** – **21**, which have high affinity and selectivity for all SRIF receptor subtypes [74,75]. The subtype 2-selective compound L-779976 potently inhibited forskolin-induced cAMP accumulation with an IC_{50} of 0.05 µM in CHO-K1 cells. This compound showed comparable potency to SRIF-14 in the inhibition of GH release from rat pituitary cells. Also, compound **18** inhibited arginine-stimulated glucagon release at a concentration of approximately 1000-fold less than blockade of insulin release. The sst_5-selective compound L-817818 was effective in blocking insulin release in mouse pancreatic islets, but this compound was inactive in the inhibition of glucagon release. The β-Trp analogue (**22**, L-054522) displayed high affinity and selectivity at sst_2 receptors; however, a major limitation of this compound is its low oral bioavailability [76]. The Merck group postulated that elimination of the hydrogen bonding ability of the urea moiety through a two carbon tether would increase the bioavailability of analogues related in structure to L-054522. This approach led to the discovery of imidazolidin-2-one **23**, a compound with an oral bioavailability of 64% [77].

Souers *et al.* [78] utilised a heterocyclic scaffold to mimic the β-turn of SRIF. Using this approach, all possible combinations of the Trp8 and Lys9 side chains of SRIF were attached to the scaffold to give a number of compounds with high affinity for ssts. Compound **24** exhibited an IC_{50} of 87 nM at sst_5 receptors.

Parallel synthesis was utilised to prepare a variety of imidazopyrazines and dihydroimidazopyrazines for SRIF receptor binding affinity in CHO-K1 cells [79]. The imidazopyrazine **25** exhibited moderate affinity (K_i of 360 ± 21 nM) at sst_5, and this derivative was shown to be an agonist in the inhibition of forskolin-induced cAMP accumulation in CHO-K1 cells expressing sst_5 receptors. These same workers prepared additional 4-phenylimidazoles bearing a D-Trp *tert*-butyl ester group at the 2-position of the imidazole ring. Compound **26** was shown to have moderate affinity for subtype 3 receptors [80]. SAR studies on the sst_3 agonist **26** led to a series of related β-carboline analogues. Compound **27** was claimed to bind at ssts; however, no functional data were presented [105]. Related 4-phenylimidazoles, compounds **28** and **29**, were described in two SCRAS patents [106,107]. The compounds were evaluated for sst_1 – sst_5 receptor binding affinity using [^{125}ITyr11]-SRIF and agonist activity by inhibition of forskolin-induced cAMP accumulation; however, no specific data were given. Numerous other analogues related in structure to compounds **25** and **26** are reported in this SCRAS patent [107]. The tetrahydroimidazo[1,2-a]pyrazine **30** is a representative of the compounds described in this patent.

In a 2001 patent [108], scientists at Novartis described the synthesis of numerous hydantoins as SRIF receptor ligands. The trisubstituted-hydantoin **31** was claimed to be an sst_2 agonist. This analogue inhibited *in vitro* GH release in cultured pituitary cells and decreased serum GH and insulin levels in rats. In addition, compound **31** exhibited oral activity in models to predict anxiolytic, antidepressant, antipsychotic and anticonvulsant activity.

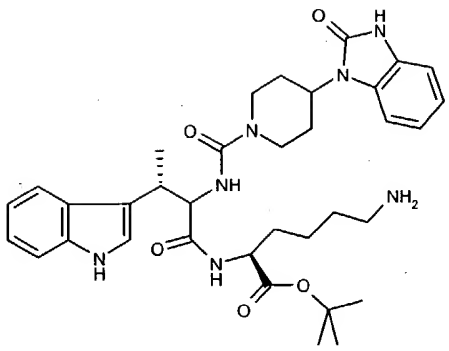
2.4 Non-peptide antagonists

The pseudosymmetry of the D-glucose moiety in compound **11** was proposed as the reason for the ability of this analogue to bind at several G-protein-coupled receptors [68]. The pseudosymmetry of the D-glucose group allows the sugar the freedom to adopt a variety of binding modes, therefore providing affinity for multiple G-protein-coupled receptors. Rohrer *et al.* [81] suggested that the D-glucose derivative **11** and related analogues bind with the precoupled form of ssts. This activated form is thought to be responsible for agonist activity in most receptor–ligand binding interactions at ssts. This hypothesis is supported by the fact that only a few SRIF antagonists are known.

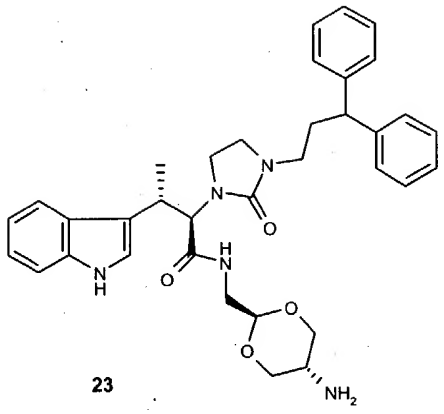
Researchers at Takeda Chemical Industries [109] described a series of tetrahydroquinolines as either SRIF agonists or antagonists. The phenylpiperazine **32** exhibited IC_{50} values of 9 and 0.8 nM, respectively, in displacement of [^{125}ITyr11]-SRIF in CHO-K1 cells at sst_2 and sst_3, however, no functional data were presented to classify this compound as an agonist or an antagonist. A subsequent patent from Takeda [110] described related derivatives. The *bis*-indolyltetrahydroquinoline **33** demonstrated IC_{50} values of 0.3, 80 and 400 nM at sst_2, sst_3 and sst_4, respectively.

Patents filed by workers at SCRAS [111,112] described the synthesis of 2-arylimino-2,3-dihydrothiazole derivatives as ligands for ssts. Thiazoles **34** and **35** were reported to have K_i values of < 200 nM in displacement of [^{125}ITyr11]-SRIF-14 in CHO-K1 cells expressing ssts.

β-Carbolines, related in structure to compound **27**, were reported in a Novartis patent [113]. These derivatives exhibit high binding affinity for sst_3 receptors and function as antagonists. The 4-phenylimidazo-β-carboline **36** was shown to have pK_d ($-\log_{10}$ dissociation constant) values of 8.69 and 8.30 nM for human and mouse subtype 3 receptors, respectively. This compound demonstrated over 400-fold greater selectivity at sst_3 receptors compared with other SRIF receptor subtypes, and compound **36** and



22 L-054522



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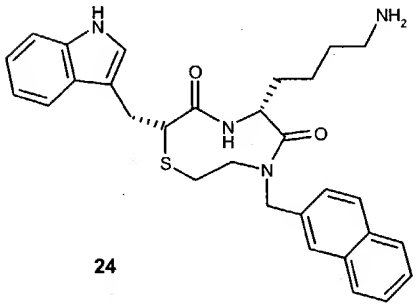

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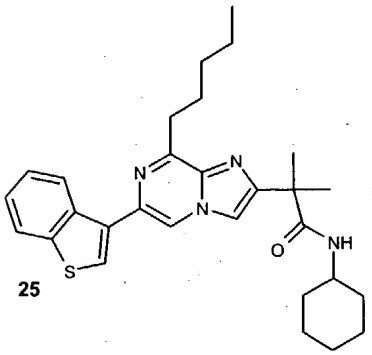

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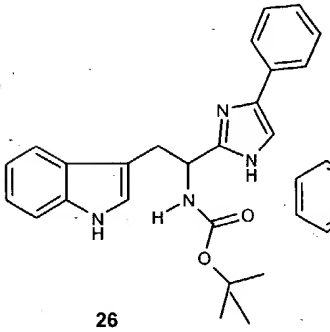

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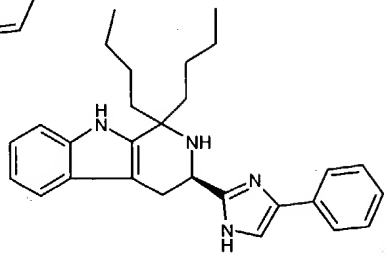

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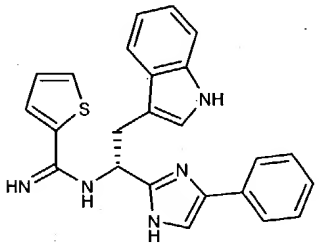

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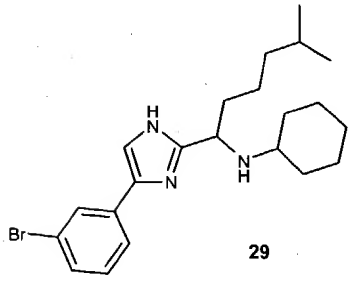

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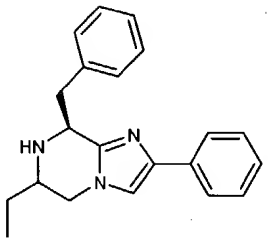

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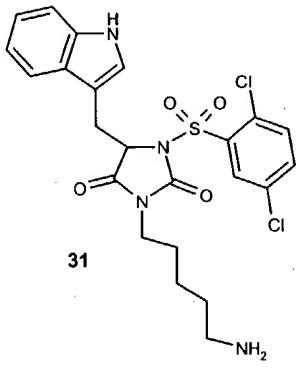

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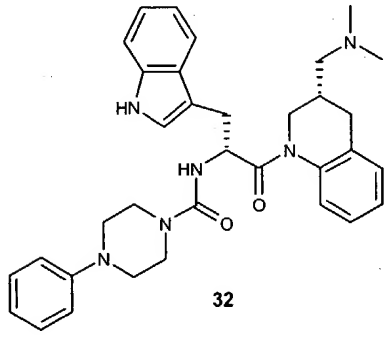

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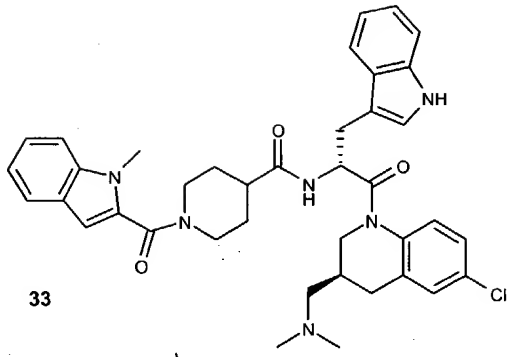

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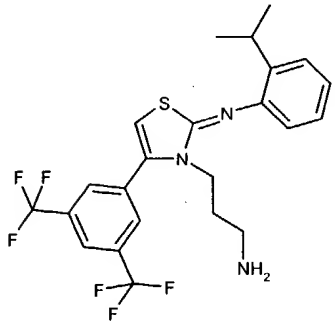

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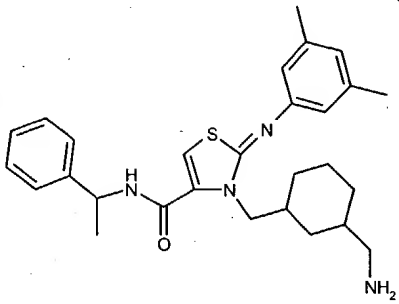

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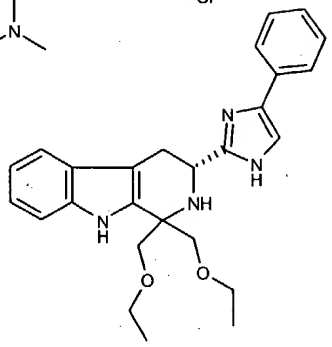

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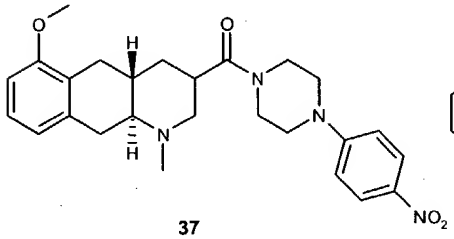

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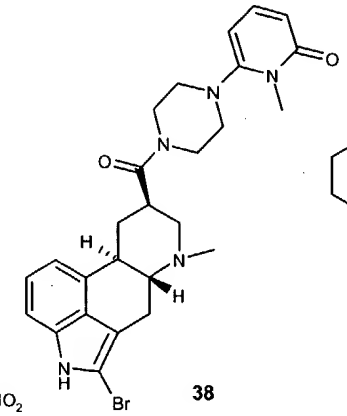

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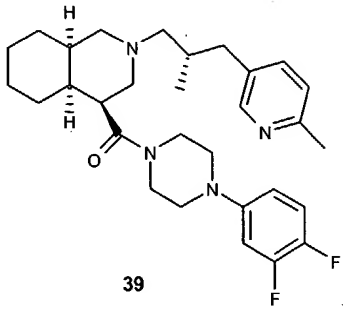

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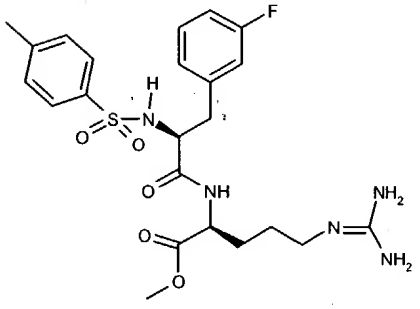

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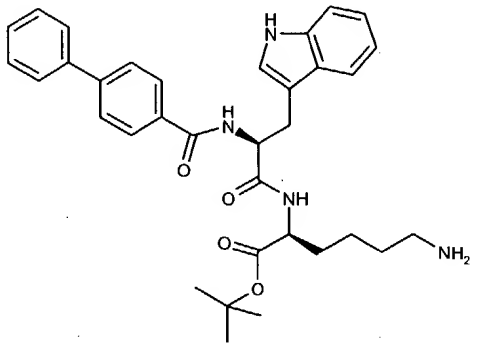

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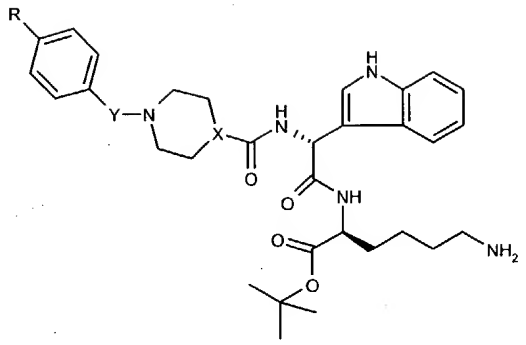
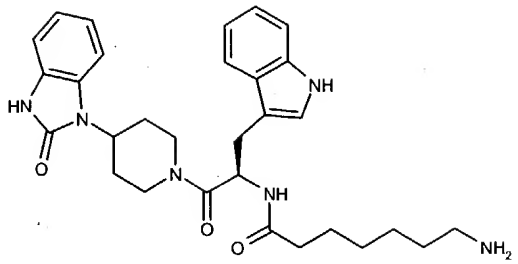


42 X = N, Y = SO_2, R = H; IC_{50} = 2.9 nM
43 X = N, Y = SO_2, R = CH_3; IC_{50} = 9.2 nM
44 X = N, Y = CO, R = H; IC_{50} = 6.9 nM
45 X = N, Y = CO, R = CH_3; IC_{50} = 4.4 nM
46 X = CH_3, Y = SO_2, R = H; IC_{50} = 3.2 nM



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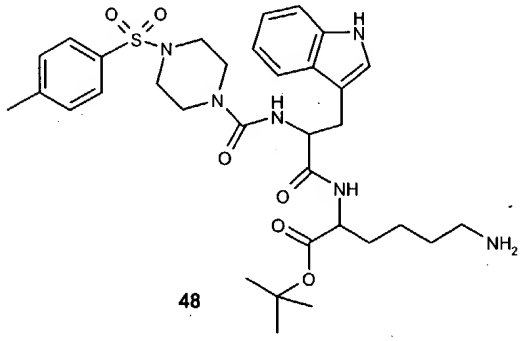

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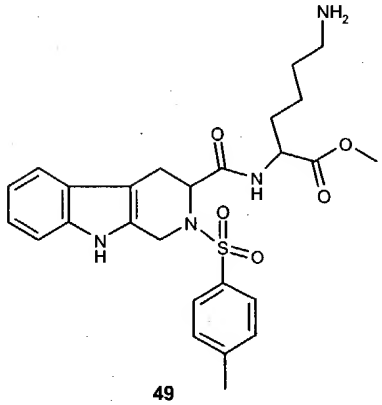

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related analogues were evaluated for CNS activity in a variety of animal models.

A Novartis patent [114] described the synthesis of octahydrobenzo[g]quinolines as selective sst_1 antagonists. The 4-phenylpiperazino-octahydrobenzo[g]quinoline **37** exhibited high affinity for native rat subtype 1 receptors (pIC_{50} = 9.1) with little affinity for other receptor subtypes. This compound was active in animal models that predict anxiolytic, antipsychotic and affective disorders such as mania. A Novartis patent [115] claimed that piperazineamides were sst_1 antagonists. The ergoline **38** exhibited a pIC_{50} value of 9.7 at native rat subtype 1 receptors and pIC_{50} values of 9 and 8.8 at native and recombinant human sst_1 receptors. This compound lowered aggressive behaviour in the matched pairs situation test and reversed social withdrawal in the intruder mouse test. Activity in these tests indicated therapeutic potential for anxiety, psychosis and mania. Decahydroisoquinolines that incorporate the 4-phenylpiperazinocarbonyl moiety at the 4-position were described in another 2001 Novartis patent [116]. Compound **39** was described as an sst_3 antagonist with activity in several tests that predict CNS activity.

Using the initial lead molecule **40**, researchers at Pfizer developed the D-Trp analogue **41** as the first non-peptide sst_2 antagonist [82]. These workers found that small structural changes in these molecules can convert antagonists into agonists. On the basis of this work, the Pfizer group modified the Merck sst_2 agonist L-054522 (**22**) in an attempt to find related sst_2 antagonists. These workers used the structural features found in L-054522 to retain potency but incorporated the structural components of **40** to impart antagonist activity. The result of this approach led to the full sst_2 antagonists **42** – **46**. Antagonist activity of these compounds was determined in pituitary cells in which cAMP content was measured. The synthesis of other close analogues of L-054522 was described in a Pfizer patent application in 2002 [117]. Representative compounds include the benzimidazol-2-one **47**, which is presumably an sst_2 antagonist. These compounds apparently increase the secretion of GH in small pigs. Other related analogues (**48** and **49**) were also described in Pfizer 2002 patent applications [118-119]. These analogues are reportedly sst_2 antagonists.

A re-evaluation of the radioligand binding and functional characteristics of the Merck agonist L-054522 and the Pfizer antagonists **42** and **46** was performed by researchers at Novartis [83]. In this study, all three compounds demonstrated agonist activity in the inhibition of forskolin-induced cAMP accumulation and the increase in luciferase reporter gene expression in CHO cells. The results of the binding studies revealed that L-054522 was 10- to 100-fold more potent than **42** and **46** in the displacement of radioligands from rat cortex

and human sst_2 receptors, which were expressed in CCL-39 cells. Furthermore, these compounds were also shown to bind with high affinity at sst_5.

3. Expert opinion

SRIF is widely distributed in the CNS, the periphery and in various tumour cells. The two biologically active forms, SRIF-14 and SRIF-28, elicit their effects through five subtypes (sst_1 – sst_5) of G-protein-coupled receptors. However, most cells contain more than one receptor subtype. Furthermore, homodimerisation and heterodimerisation of SRIF receptors may alter receptor function. The closely related peptide CST binds equally well to SRIF receptor subtypes. Whether specific CST receptors exist remains to be determined. Peptide analogues have been discovered by peptide backbone modification and truncation; however, the problems of enzymatic liability and suitable delivery forms of these analogues present major challenges. The work of Merck scientists that led to the development of SRIF subtype-specific non-peptide agonists was a significant achievement. Furthermore, the discovery of non-peptide antagonists by researchers at Pfizer will facilitate the study of the role and function of individual SRIF receptor subtypes. However, additional research is necessary to fully characterise these novel antagonists. Peptide analogues such as octreotide have demonstrated clinical effectiveness in the treatment of acromegaly and various tumours. Other potential therapeutic uses of SRIF analogues include treatment of disorders of the CNS, modulation of glucose homeostasis, regulation of insulin and glucagon secretion, tumour diagnosis and treatment and diabetic retinopathy.

In a very short time, subtype-selective ligands have been discovered for all SRIF receptor subtypes. Many of these compounds offer new options in the treatment of various disease states. In addition, these compounds provide valuable tools to study the role and function of SRIF and its receptors throughout the body. Research on SRIF should continue to be a fertile area of study for the future.

Acknowledgements

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Patents

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104. TULANE EDUCATIONAL FUND: WO0272602 (2002).

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Affiliation

A Michael Crider
School of Pharmacy,
University of Louisiana at Monroe, Monroe,
LA 71209-0470, USA
Tel: +1 318 342 1707; E-mail: crider@ulm.edu